Acidified sodium chlorite as an alternative to chlorine to control microbial growth on shredded carrots while maintaining quality

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Abstract: Shredded carrots are particularly susceptible to microbial growth and quality deterioration as a result of a large cut surface area to mass ratio. Acidified sodium chlorite (ASC) in the concentration range 500–1200 μL L^{-1} has been shown to have stronger efficacy against pathogens and spoilage bacteria than chlorine and does not form carcinogenic products. However, ASC in this concentration range aggravates tissue damage. The objective of this study was to optimize ASC treatment parameters to balance antimicrobial activity with quality retention of shredded carrots. Shredded carrots were immersed for either 1 min in 100, 250 or 500 μL L^{-1} ASC solutions or 2 min in 200 μL L^{-1} chlorine or water (control). Treated samples were spin-dried and packaged in polypropylene bags and stored at 5 °C for up to 21 days. Carrots were evaluated at 7-day intervals for visual appearance, package atmosphere composition (O_2 and CO_2), product firmness, tissue electrolyte leakage and pH. The microbial growth, including total aerobic bacterial counts, total coliforms/*Escherichia coli*, yeast and mold counts and lactic acid bacterial counts on the products was also determined. Treatments with all concentrations of ASC reduced the aerobic bacterial counts, coliform/*E. coli* counts, yeast mold and counts and lactic acid bacterial populations by 1.2–2.0 log cfu g^{-1} when compared with the water-washed and unwashed samples. During storage, unwashed samples had a sharp increase in lactic acid bacterial populations accompanied by a sharp decline in pH readings and rapid loss in firmness and tissue integrity; samples washed with 100 μL L^{-1} ASC maintained the best overall visual quality, accompanied by the retention of tissue integrity and firmness. Therefore, 100 μL L^{-1} was determined as the optimum concentration of ASC for maintaining overall quality and firmness, inhibiting microbial growth and prolonging the shelf-life of shredded carrots.

Keywords: acidified sodium chlorite; microbial growth; shelf-life; shredded carrots

INTRODUCTION

The minimally processed fresh-cut produce industry has been growing rapidly over the past decade, fueled by strong consumer demand for ready-to-eat produce that is convenient and nutritious.[1] However, rapid quality deterioration and short shelf-life are major problems facing the industry, making necessary the development of new technologies for maintaining the quality and safety of fresh-cut produce while prolonging shelf-life. Although modified atmosphere packaging (MAP) has been successfully used to improve quality and shelf-life, premature quality decline and the consequent loss of shelf-life remain important obstacles to be overcome.[2] Quality deterioration in fresh-cut carrots is due to the development of whiteness, tissue softening, off-odor and tissue decay.[3]

Research studies have shown that fresh-cut products are particularly susceptible to microbial growth owing to the removal of plant protective tissues (skins, etc.) and the release of cellular fluids from cutting.[3,4] The large exposed surface area of shredded carrots causes them to be particularly vulnerable to microbial attack, moisture loss and dehydration. Washing is an important step that has been widely adopted by the industry to remove microorganisms, tissue fluids and other foreign materials.[2] However, cross-contamination of produce with microorganisms, including human pathogens, can occur during produce washing. The development and proper application of sanitizing agents to remove microorganisms and control pathogen cross-contamination effectively is critical to ensure the quality and safety of fresh-cut produce.

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Chlorine (100–200 $\mu L\,L^{-1}$) has been widely used as a sanitizer during produce washing.[5–7] However, numerous studies have shown that chlorine used at concentrations permitted by the FDA lacks efficacy in removing human pathogens and spoilage microorganisms.[7,8] Additionally, chlorine may react with organic matter in water to form carcinogenic products.[9] The inadequacies of chlorine as a sanitizer have stimulated interest in finding safer, more effective sanitizers.[10]

Acidified sodium chlorite (ASC) (Alcide, Redmond, WA, USA) (500–1200 $\mu L\,L^{-1}$) has recently been approved by the FDA for spray or dip application on various food products, including fresh and fresh-cut produce.[11] ASC is a mixture of citric acid and sodium chlorite in aqueous solution, which has strong antimicrobial properties primarily owing to its oxidative mode of action. ASC has a substantial antimicrobial effect against *Escherichia coli* O157:H7 and *Salmonella* spp. inoculated on to cantaloupes, honeydew melons and asparagus spears with pathogen reductions of 3 log cfu g^{-1}.[12] Our earlier studies have shown that ASC had a strong efficacy on pathogen inactivation with up to 5 log cfu g^{-1} reduction on *E. coli* O157:H7 inoculated on shredded carrots tested under simulated commercial fresh-cut produce wash conditions;[13] *E. coli* O157:H7 was not recovered on the carrots treated with ASC during the entire 14-day storage; enrichment studies also did not recover any *E. coli* O157:H7. However, tissue injury manifested as tissue softening was apparent on shredded carrots treated with ASC at the test concentration and duration of immersion (1000 $\mu L\,L^{-1}$, 2 min). The main objective of this study was to optimize ASC treatment concentration to be effective for microbial reduction while maintaining tissue integrity and prolonging shelf-life of shredded carrots.

MATERIALS AND METHODS

Sample preparation

Carrot (*Daucus carota*, var. *sativus*) roots were purchased from a local wholesale market in Jessup, MD, USA and used within 24 h following storage at 5 °C. Carrot roots with visible damage were removed. Carrot samples were washed with tap water to remove residual soil and then shredded with a domestic food processor (Cuisinart, East Windsor, NJ. USA). The carrot shreds were divided into individual 1000-g portions contained in nylon mesh bags and washed in the selected sanitizer solutions as described below.

Treatment procedure

Shredded carrots were submerged in solutions containing 200 $\mu L\,L^{-1}$ of chlorine with the pH adjusted to 6.5 with HCl or 100, 250 or 500 $\mu L\,L^{-1}$ ASC. Sodium chlorite was acidified with citric acid following the manufacturer's recommendations. The final pH of 100, 250 and 500 $\mu L\,L^{-1}$ solutions was 2.71, 2.55 and 2.47, respectively. The unwashed samples and samples washed in tap water were included as controls. The carrot to solution ratio in all treatments was 1:10 (w/v). The immersion duration was 1 min for all ASC treatments and 2 min for chlorine and water. After dipping, shredded carrots were centrifuged for 2 min (650 rpm) to remove excess water, using a commercial salad centrifugal dryer (Model T-304, Garroute Spin Dryer, Meyer Machine, San Antonio, TX, USA). Samples of 200 g each were packaged in polypropylene bags (18 × 22 cm) with an oxygen transmission rate of 290 mL day^{-1} m^{-2} O_2 and sealed using a PFS-F450 impulse sealer (Kingstar Manufacturing, Wenzhou, China). Samples were inspected for potential gas leakage before storage. All samples were stored at 5 °C for up to 21 days for subsequent quality and microbial evaluation at 7-day intervals.

Atmosphere composition measurement

Gas samples were withdrawn from the packages through a septum using a gas-tight hypodermic syringe. The O_2 and CO_2 partial pressures (kPa) were determined using an O_2/CO_2 infrared gas analyzer (Model S-3A/I and Model CD-3A, Ametek, Pittsburgh, PA, USA).

Tissue electrolyte leakage analysis

Tissue electrolyte leakage was measured following a modified procedure of Luo *et al.*[14] Each 50-g sample was submerged in 500 mL of deionized water at 5 °C for 30 min. The electrical conductivity (μS) of the solution was measured using a conductivity meter (Model AB30 Accumet Basic, Fisher Scientific Co., Pittsburgh, PA, USA). Total electrolytes of the samples were determined after freezing the samples at 20 °C for 24 h and subsequently thawing to room temperature. Relative conductivity was expressed as a ratio of the 30-min conductivity measurement over total electrolytes.

Firmness, expressed juice, pH and overall quality determination

A TA-XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY, USA) equipped with a Kramer shear press possessing 10 blades was used to measure the shear force (kN) required to shear 50 g of shredded carrots at a crosshead speed of 10 mm s^{-1} and a full scale of penetration of 20 mm. Maximum force was recorded and used to indicate firmness of carrot samples.

The expressed juice was measured following the procedure of Carlin *et al.*[15] Samples of 10 g each were placed between two pre-weighed No. 1 filter-papers of 10 cm diameter (Whatman, Maidstone, Kent, UK). A 10-kg weight was placed on the filter-paper for 10 s and then the filter-papers were re-weighed. Values were reported in grams of expressed liquid per 100 g fresh sample.

The pH was measured following a modified procedure of Barry-Ryan *et al.*[16] Samples weighing

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50 g each were blended in 50 mL of deionized water for 1 min in a domestic blender (Waring Products, Torrington, CT, USA). The pH of the macerate was determined using a digital pH-meter (Oakton Instruments, Vernon Hills, IL, USA).

The overall visual quality of shredded carrots was evaluated following a modified procedure of Cantwell *et al*.[17] and Mercado-Silva *et al*.[18] by a four-member trained panel. Before the test, the panel members were trained to recognize and scale the quality attributes of shredded carrots. Samples were coded with random three-digit numbers to mask the treatment identity. The acceptability was scored using a nine-point hedonic scale, where 9 = excellent, no defects, 7 = very good, minor defects, 5 = fair, moderate defects, 3 = poor, major defects and 1 = unusable.[17] Minor defects were attributed to dryness of the surface tissue and major defects were usually due to decay. A score of 5 or below was considered to be unsaleable.

Microbial enumeration

Shredded carrot samples (30 g) were macerated in sterile peptone water using a stomacher blender (Model 400, Seward, London, UK) for 2 min at 230 rpm and filtered through sterile glass-wool. The homogenate and its serial dilutions were logarithmically spread on agar plates with an automatic spiral plater (Wasp II Spiral Plater, DW Scientific, Shipley, West Yorkshire, UK). Enumeration of the selected microorganisms was performed using the following culture media and conditions according to Allende *et al*.:[19] (1) Tryptic Soy Agar (Difco Laboratories, Sparks, MD, USA) incubated aerobically at 28 °C for 24–48 h for total aerobic bacteria; (2) Potato Dextrose Agar (Difco Laboratories) supplemented with 300 μg ml^{-1} chloramphenicol (Clr, Difco Laboratories) incubated at 25 °C for 5 days for yeast and molds; (3) Lactobacilli Man–Rogosa–Sharpe agar (Difco Laboratories) incubated at 35 °C for 72 h under 20 kPa CO_2 and 5 kPa O_2 provided with a water-jacketed incubator with automatic gas control (Forma Scientific, Marjetta, OH, USA) for lactic acid bacteria; and (4) 3M^{TM} coliforms/*E. coli* Petrifilm (3M, St Paul, MN, USA) spread and incubated at 35 °C for 24 h for coliforms/*E. coli*. Microbial colonies were counted using a Protos Colony Counter (Model 50 000, Synoptics, Cambridge, UK) and reported as log cfu g^{-1} tissue.

Statistical analysis

Experiments were repeated twice, each with three replications. Results of the two experiments were combined for a total of six replications. Analysis of variance (ANOVA), followed by Tukey's multiple range test for comparison of means and least significant differences (LSD) at $\alpha = 0.05$, were performed using the Number Cruncher Statistical Systems'97 (NCSS97) Statistical Software, version 6.0 (NCSS, Kaysville, UT, USA). Unless stated otherwise, only results significant at $P \leq 0.05$ are discussed.

RESULTS AND DISCUSSION

Package atmospheres and product quality

Package atmospheres

A modified atmosphere within the bags was formed owing to the consumption of O_2 and production of CO_2 via the respiration process of shredded carrots and the gas transmission through the package film. Table 1 illustrates the evolution of the gas compositions during storage of packaged shredded carrots washed with water, chlorine and ASC. The oxygen partial pressures inside the packages of all treatments decreased rapidly upon packaging and reached equilibrium of 0.3–0.9 kPa O_2 by day 7 (Table 1). No significant difference between O_2 partial pressures was found among treatments. Carbon dioxide partial pressures showed a corresponding rapid increase from day 0 to day 7 and remained stable during the remainder of the storage. Among all treatments, the unwashed control had significantly higher CO_2 partial pressures on day 14 (21 kPa) than the other treatments. The lowest CO_2 accumulation was observed in the packages containing shredded carrots washed with 100 μL L^{-1} ASC. The unwashed carrot shreds increased in oxygen and decreased in carbon dioxide at the end of storage, very likely as the result of a significant reduction in respiration rate caused by senescence and cell death. In addition, the accumulation of lactic acid and reduction in pH (Fig. 1) may have caused the degeneration of lactic

Table 1. Effect of sanitizer treatment on O_2 and CO_2 partial pressures of packaged shredded carrots stored at 5 °C for up to 21 days[a]

	O_2 (kPa)				CO_2 (kPa)			
Treatment	0 days	7 days	14 days	21 days	0 days	7 days	14 days	21 days
Unwashed	21a	0.28c	0.13a	1.49a	0.03a	17.50a	21.00a	14.79a
Water	21a	0.21d	0.14a	0.09b	0.03a	15.99b	15.99b	12.91b
Chlorine	21a	0.47b	0.17a	0.10b	0.03a	14.13c	15.55b	12.89b
100 μL L^{-1} ASC	21a	0.52b	0.17a	0.12b	0.03a	13.57d	13.28c	11.92c
250 μL L^{-1} ASC	21a	0.88a	0.15a	0.10b	0.03a	13.20d	15.49b	13.00b
500 μL L^{-1} ASC	21a	0.82a	0.14a	0.09b	0.03a	13.57d	15.42b	13.37b

[a] Means with the same letters during the same period of storage are not significantly different according to the Tukey test ($P > 0.05$).

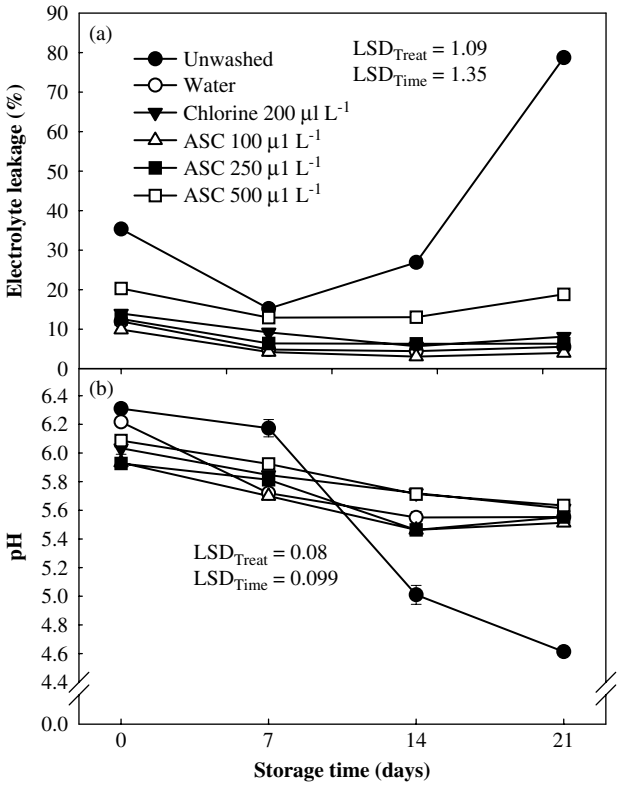

Figure 1. Effect of sanitizer treatment on tissue electrolyte leakage (a) and pH (b) of packaged shredded carrots stored at 5 °C for up to 21 days. Each symbol is the mean of six replications; vertical lines represent SE. SE lines are not shown when masked by the symbol.

acid bacteria and the decrease in metabolism and production of carbon dioxide as a result of negative feedback regulation.

Changes in postharvest physiology and product quality

Electrolyte leakage has often been used as an indicator of produce quality and freshness.[14] Tissue electrolyte leakage is assessed by measuring the electrical conductivity (EC) of water in which samples have been immersed for a period of time. Low EC values are obtained when membranes are intact.[20] As shown in Fig. 1(a), carrot samples washed with 100–250 µL L^{-1} ASC, 200 µL L^{-1} chlorine and water maintained a low tissue electrolyte leakage throughout the storage, suggesting little deterioration of carrot samples subjected to these treatments. However, carrot samples treated with 500 µL L^{-1} ASC showed significantly more electrolyte leakage than the other samples, probably resulting from tissue damage caused by ASC treatment at high concentration. Samples packaged without the washing step had the highest electrolyte leakage rate at the beginning of the storage owing to the residual carrot juice and subsequently exhibited a sharp increase towards the end of the storage, probably owing to the tissue breakdown caused by microbial growth.

The initial pH ranged from 5.9 to 6.1 after washing with chlorine or ASC and measured 6.3 when washed with water or unwashed [Fig. 1(b)]. Similar results for initial pH were reported by Izumi and Watada[21] and Barry-Ryan *et al.*[16] The pH decreased slowly during storage to levels of 5.4–5.6 in all treatments without significant differences, except for unwashed shredded carrots, which experienced a sharp decline in pH beginning on day 7 and reaching a value of 4.4 by the end of day 21 of storage. The pH decrease could be attributed to the acid produced from fermentation in response to high CO_2,[22] as evidenced by the large population of lactic acid bacteria present on this treatment. Similar findings were reported by Izumi and Watada[21] and Barry-Ryan *et al.*[16]

The firmness of carrot shreds was similar for all treatments according to firmness readings measured immediately following treatment on day 0 [Fig. 2(a)]. In general, there was a slight increase in firmness readings from day 0 to 7, followed by relatively stable firmness readings during the remainder of the storage for most samples. However, firmness readings on samples treated with 500 µL L^{-1} ASC were lower than those on other sanitizer treatments. There was a steady decline in firmness of unwashed samples from day 7 to day 21. Carrots washed with water showed the highest increase in firmness value during storage, probably owing to the drying out of the surface tissues of carrots. Water–washed carrots exhibited the major symptoms of surface dehydration including the physiological defect of 'whitening', which is caused by the formation of lignin.[23–25] Expressed juice, which has been used as a measurement of freshness,[26,27] is determined as a percentage by mass of cellular sap released per 100 g of carrot. Initial expressed juice ranged from 1.8 to 2.2% with no significant difference among treatments [Fig. 2(b)]. The expressed juice from all treatments, except the unwashed control, remained stable during storage after a slight decline from day 0 to day 7. The rapid increase in the expressed juice from the unwashed control may indicate a rapid cellular breakdown, which was consistent with the quality deterioration observed in this treatment.

Overall visual quality rating of samples treated with 100 µL L^{-1} ASC was significantly higher than for other treatments throughout the storage (Fig. 3), followed by those washed with chlorine and 250 and 500 µl L^{-1} ASC. Unwashed samples had the lowest scores from day 14 until the end of storage. The low quality score in this treatment was mainly due to the decayed and slimy appearance of samples. The changes in firmness, expressed juice and electrolyte leakage agreed with visual evaluation result that 100 µL L^{-1} ASC was the preferred treatment followed by 250 and 500 µL L^{-1} ASC. Samples washed with water also received a significantly lower quality score, mainly owing to the appearance of whitening caused by the drying out and lignification of tissues. Higher firmness readings in these samples confirmed this assessment.

Microbial growth

A wide variety of microorganisms have been found on fruits and vegetables. These include mesophilic bacteria, lactic acid bacteria, coliforms, yeasts and

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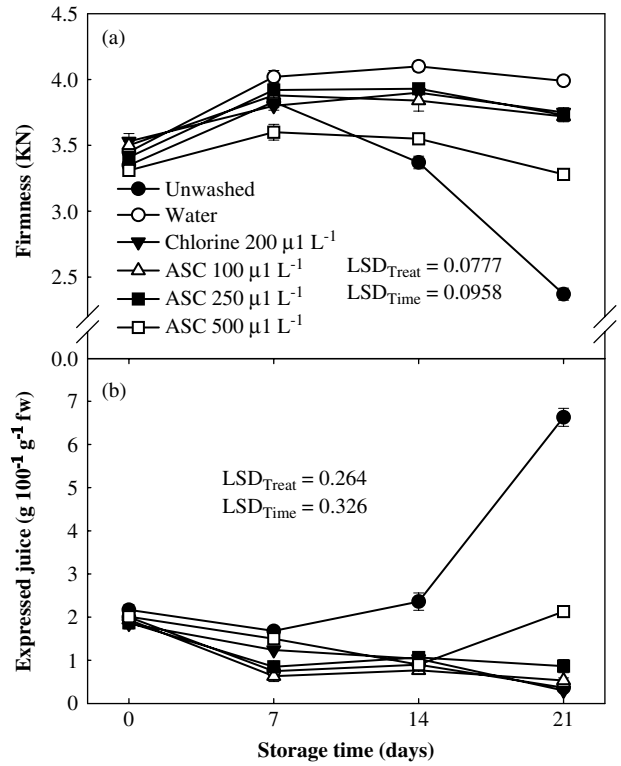

Figure 2. Effect of sanitizer treatment on firmness (a), expressed as kilonewtons (kN) and expressed juice (b), in g per 100 g fresh weight of packaged shredded carrots stored at 5 °C for up to 21 days. Each symbol is the mean of six replications; vertical lines represent SE. SE lines are not shown when masked by the symbol.

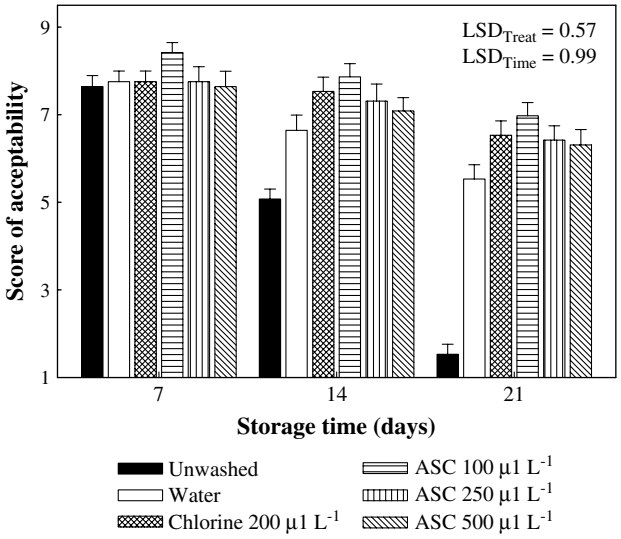

Figure 3. Effect of sanitizer treatment on overall quality of packaged shredded carrots stored at 5 °C for up to 21 days. Overall quality was scored by four trained panelists using a 1–9 hedonic scale where 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much and 9 = like extremely. Each symbol is the mean of six replications; vertical lines represent SE.

molds.[2] Zagory[28] noted that these microorganisms are ubiquitous in these produce and are sometimes associated with the quality deterioration and end of shelf-life of fruits and vegetables; therefore, the use of sanitizers to inhibit microbial growth is essential to maintain product quality and extend shelf-life. Initial populations of total aerobic bacteria [Fig. 4(a)], total coliforms [Fig. 4(b)], molds and yeasts [Fig. 5(a)] and lactic acid bacteria [Fig. 5(b)] on unwashed shredded carrots were 5.2, 4.3, 3.1 and 3 log cfu g^{-1}, respectively. These findings are in agreement with the results of Carlin *et al.*,[15] Kakiomenou *et al.*,[22] Sinigaglia *et al.*[29] and Gonzalez *et al.*[13] Treatment with ASC at all three concentrations tested significantly reduced the total aerobic bacterial count on day 0 compared with the water-washed, unwashed and chlorine-washed carrots. Although the aerobic bacteria grew rapidly during cold storage, all concentrations of ASC-treated samples maintained the lowest bacterial counts throughout the storage. Park and Beuchat[12] reported similar results on total aerobic bacterial reduction for ASC treatment of asparagus.

All three concentrations of ASC treatment and chlorine caused significant reductions in total coliform counts on day 0 compared with the water-washed and unwashed controls. This reduction was maintained throughout the storage [Fig. 4(b)]. No significant difference was observed in yeast and mold growth between any of the treatments, although significant growth was observed for all treatments from day 0 to day 7 [Fig. 5(a)]. This result is similar to that obtained in an earlier study by Gonzalez *et al.*[13] Chlorine treatment resulted in a significant

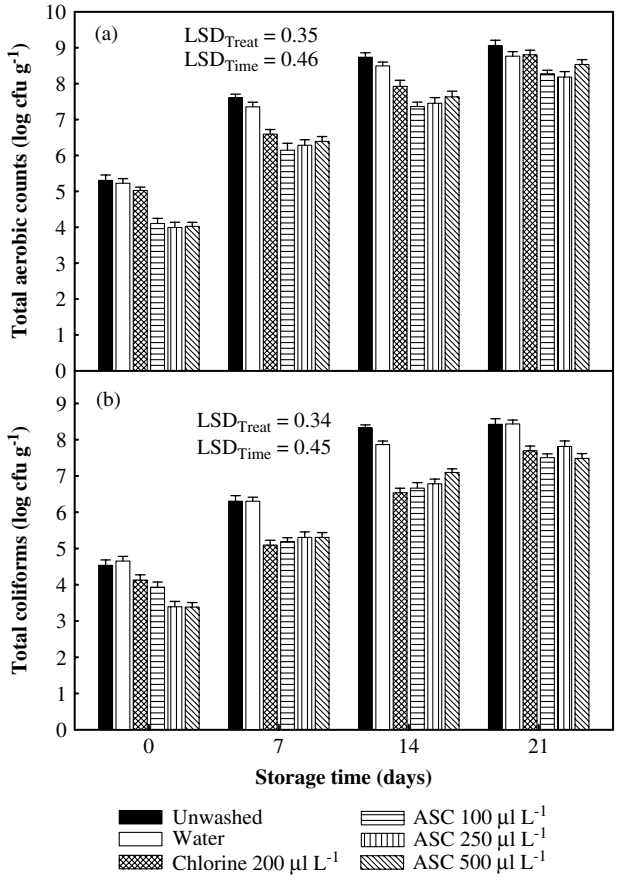

Figure 4. Effect of sanitizer treatment on the reduction of total aerobic counts (a) and total coliforms (b) in shredded carrots. Each symbol is the mean of six replications; vertical lines represent SE.

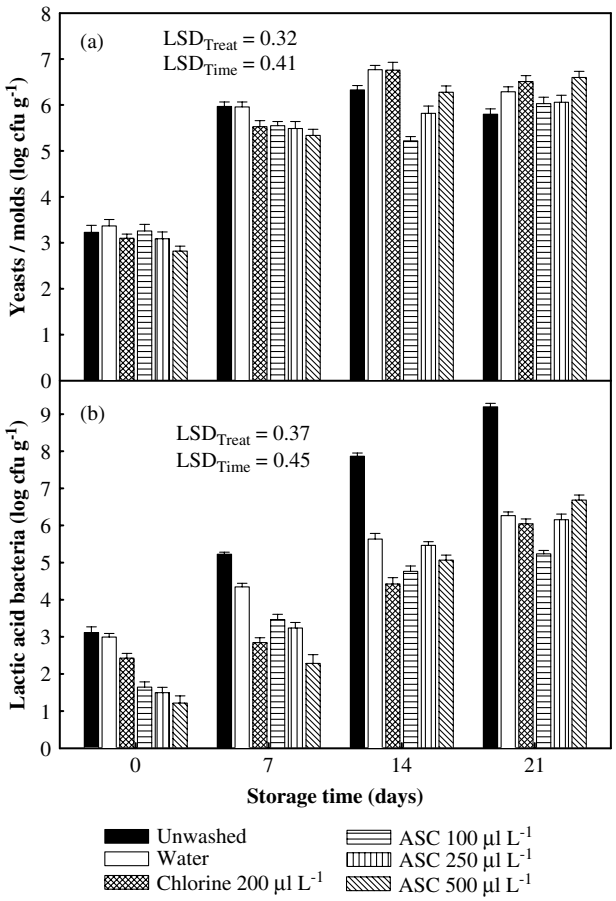

Figure 5. Effect of washing shredded carrots with different sanitizers on the reduction of yeasts and molds (a) and lactic acid bacteria (b). Each symbol is the mean of six replications; vertical lines represent SE.

reduction (0.7 log cfu g^{-1}) over the water-washed and unwashed controls on the initial counts of lactic acid bacteria (LAB) on shredded carrots on day 0 [Fig. 5(b)]. ASC treatments at all three concentrations afforded strong reductions in LAB, with about 0.75 log cfu g^{-1} reduction over chlorine treatment and 1.5–1.9 log cfu g^{-1} over controls. During storage, although LAB counts increased substantially on samples from all treatments, the increase was greatest on the unwashed samples, reaching 9.2 log cfu g^{-1} at the end of storage. This is probably due to the high sugar content in the carrot juice, which remained on the surface of the unwashed carrot samples. The high LAB count corresponds with the sharp decline in pH of the unwashed carrots samples [Fig. 1(b)]. Among all treatments, application of 100 µL L^{-1}ASC seemed to result in the lowest LAB counts towards the end of the storage. The antibacterial activity capacity of ASC is attributed to the chlorous acids which is formed by the acidification of chlorite.[30] Moreover, the low pH of ASC solutions (~2.5) probably affect the cell's ability to maintain pH homeostasis, disrupting substrate transport and inhibiting metabolic pathways.[31] ASC is currently approved in the range 500–1200 µL L^{-1} by the FDA.[11] In this concentration range, ASC exhibited strong efficacy over pathogen inactivation on various food products.[11,12] However, over the 3 weeks of storage, 100 µL L^{-1} ASC not only maintained the best quality of shredded carrots, but also was more effective at controlling microbial growth than the 500 µL L^{-1} during the last 2 weeks of storage. Quality was negatively impacted by application of ASC at the approved range whether or not it was followed by a potable water rinse. Additional studies on fresh-cut lettuce and cilantro revealed a similar negative impact on product quality when ASC was applied in the approved range. Similar findings were also reported by Bosilevac *et al.*,[32] who found that red meat products treated with 300 µL L^{-1} ASC were superior to those treated with 600 µL L^{-1} ASC. Given that ASC is currently approved at 500–1200 µL L^{-1}, FDA approval for the treatment range below 500 µL L^{-1} is needed before its commercial application. In fact, EcoLab, the company that commercially produces ASC, is in the process of petitioning the FDA to remove the lower limit of the approved range, thus allowing ASC to be applied at lower concentrations with optimal quality and safety benefit, as demonstrated by this research on carrots and by Bosilevac *et al.*[32] on red meat products.

CONCLUSIONS

Significant differences in quality and microbial growth were found among carrot samples treated with different concentrations of ASC, chlorine and controls. Among all treatments, application of 100 µL L^{-1} ASC produced samples with the best overall visual quality, accompanied by the lowest aerobic bacterial counts, yeast and mold counts and LAB counts. Since the best treatment concentration 100 µL L^{-1} of ASC for produce quality found in this study is much lower than the approved range, it is expected that these findings will provide an effective antimicrobial application with ASC that is more economic, provides superior produce quality and longer shelf-life and leaves less chemical residue than the current approved range.

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